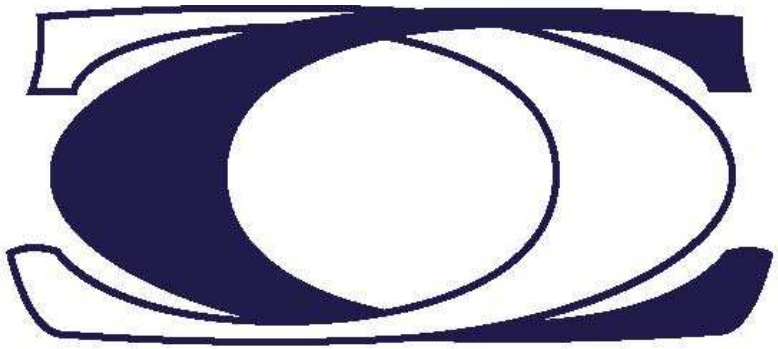

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Innovation Begins Here

On the method development of immobilized polysaccharide chiral stationary phases in SFC using extended range of co-solvents

William L. Watts[1], Jr., J.T. Lee[1],
James Lee[1], Joseph Barendt[1], Frank Riley[2] and Pilar Franco[3]

[1] CHIRAL TECHNOLOGIES INC.
[2] CHIRAL TECHNOLOGIES EUROPE
[3] PFIZER

SFC 2013

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SFC as efficient screening tool for chiral molecules

PRIMARY SCREENING

Limited selection of
Chiral Stationary Phases (CSPs)

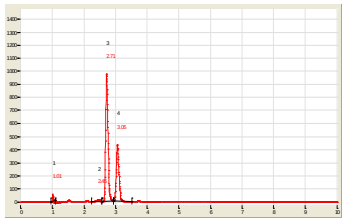
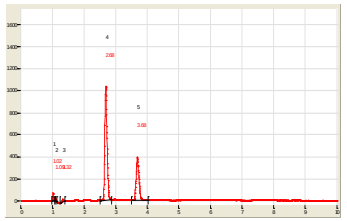
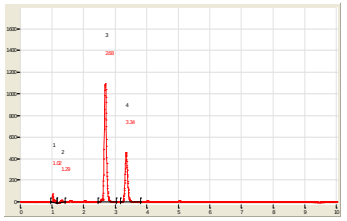
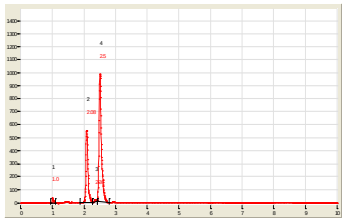
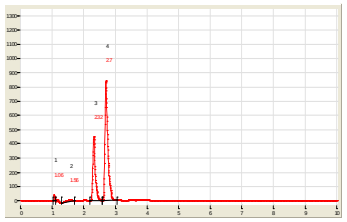
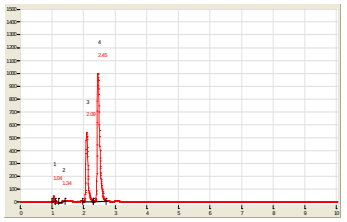
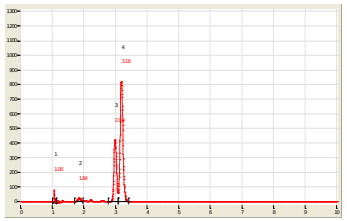
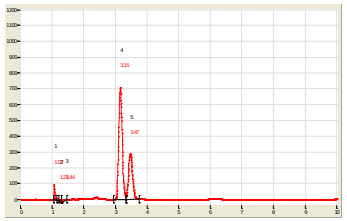
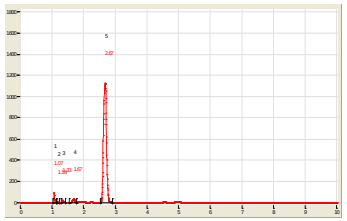
Co-solvents

EtOH, MeOH, 2-PrOH

(sometimes Acetonitrile)

Approach with high success rate!

Broadly accepted

	CO_2 / MeOH + 1%DEA 80/20	CO_2 / IPA + 1%DEA 80/20	CO_2 / EtOH + 1%DEA 80/20
IA			
IB			
IC			

How choosing the CSPs?

Broad recognition

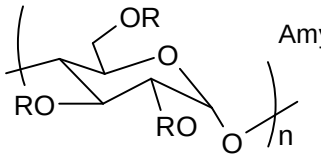
Most general purpose screening

Analytical or preparative application

Limited number of columns

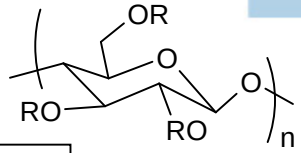
Polysaccharide-derived selectors

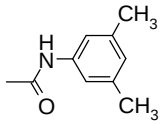
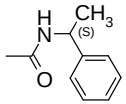
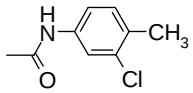
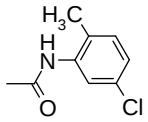
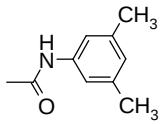
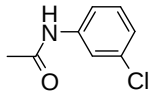
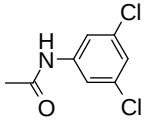
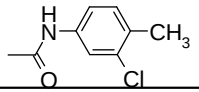
A number of CSPs available

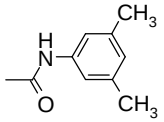
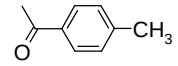
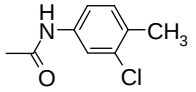
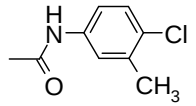
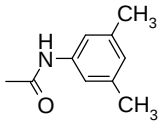
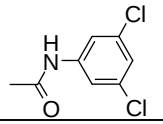


Amylose-based

Cellulose-based



CSP	Nature	-R
CHIRALPAK AD-H	Coated	
CHIRALPAK AS-H	Coated	
CHIRALPAK AZ-H	Coated	
CHIRALPAK AY-H	Coated	
CHIRALPAK IA	Immobilized	
CHIRALPAK ID	Immobilized	
CHIRALPAK IE	Immobilized	
CHIRALPAK IF	Immobilized	

CSP	Nature	-R
CHIRALCEL OD-H	Coated	
CHIRALCEL OJ-H	Coated	
CHIRALCEL OZ-H	Coated	
CHIRALCEL OX-H	Coated	
CHIRALPAK IB	Immobilized	
CHIRALPAK IC	Immobilized	

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Screening works but there are a number of aspects to be investigated

The start of the project

- CTI and Pfizer Pharmaceuticals in Groton started the first discussions about the project in 2010
- A joint project was setup to learn more about:
  - Screening strategies for the resolution of enantiomers in SFC
  - The use of extended solvent range
  - Additional options offered for prep use
- Pfizer loaned an analytical SFC to CTI for the project

Why extended solvent range in SFC could be of interest?

- ✓ Solubility reasons
- ✓ Stability issues
- ✓ Increase of screening success rate

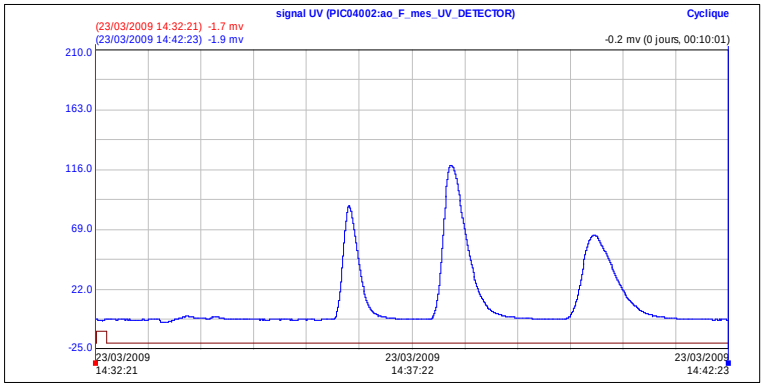
Sample solubility

CHIRALPAK IC
(250 x 30 mm)
CO_2/EtOH 70/30
120 ml/min, 25°C

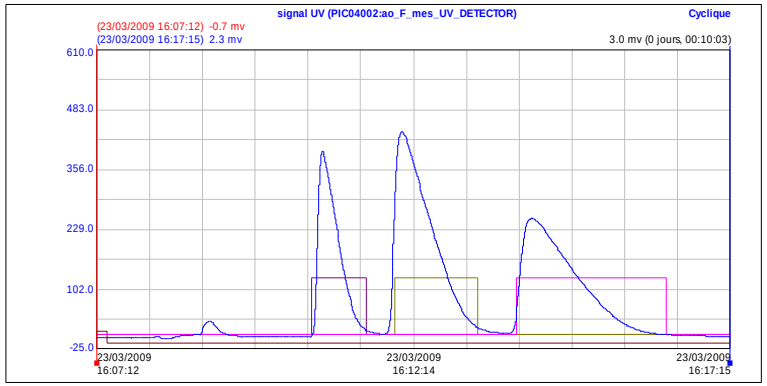
DCM is less polar than alcohols or THF
(not compatible with all columns)

Solubility in EtOH < 2 g/L
Solubility in EtOH/DCM 90/10 = 58 g/L

Injecting in a solvent different from mobile phase



Analytical injection



Injection in EtOH/DCM 90/10 – 2ml - 116 mg

No perturbation of the separation

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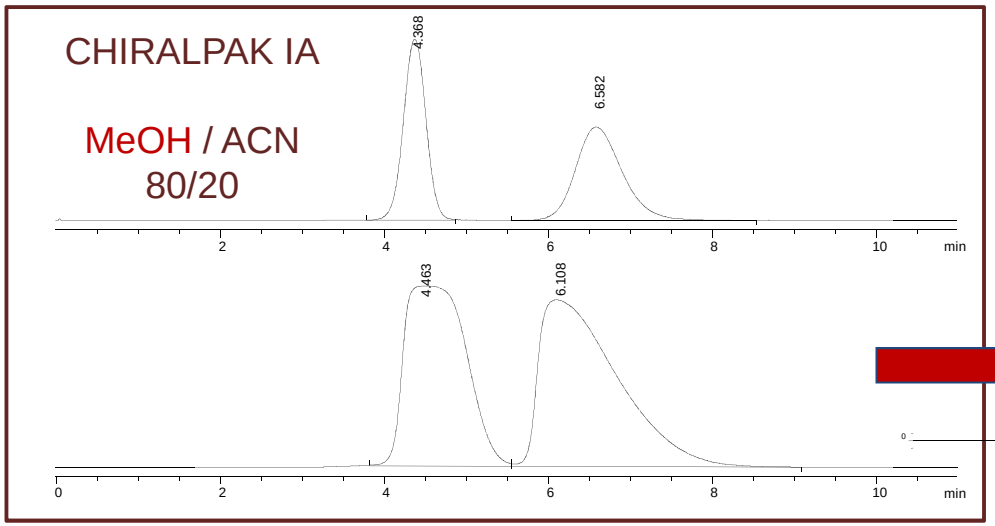
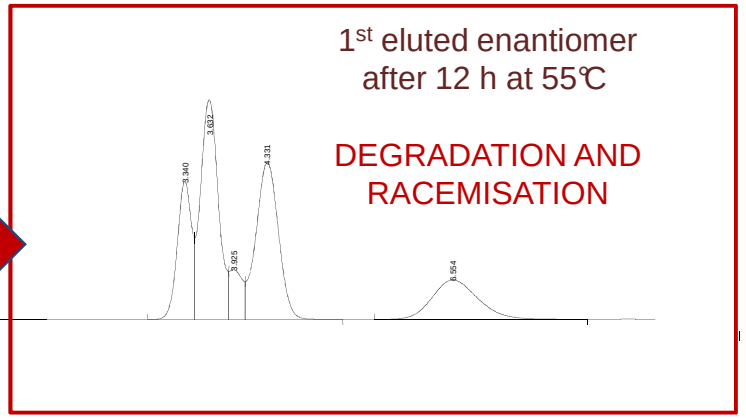
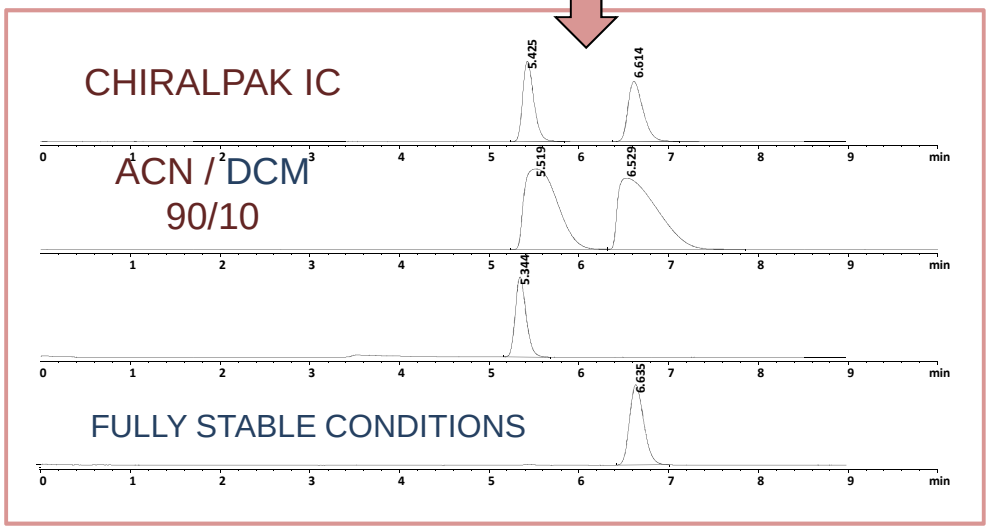
Sample stability

A case study in LC







Compound with glutarimide moiety

In certain cases, degradation and/or racemisation can be controlled with lower evaporation temperature

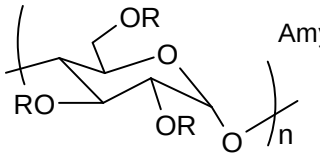
Extended Range Co-Solvent System

- ✓ Evaluations of ethyl acetate, THF and DCM
- ✓ Use of immobilized-type polysaccharide CSPs

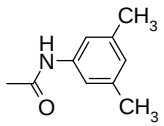
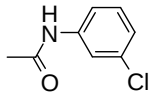
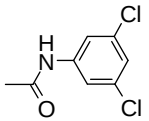
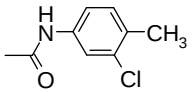
- ✓ Parameters investigated:

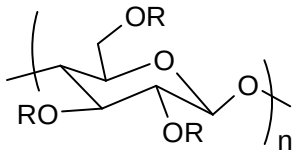
  - ✓ Elution strength of those solvents in SFC
  - ✓ Role of the addition of alcohol in the elution and the success rate
  - ✓ Role of additives for the elution and resolution of acidic and basic molecules

Structure of CSPs used in the study

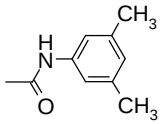
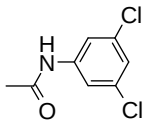


Amylose-based

CSP	Nature	-R
CHIRALPAK IA	Immobilized	
CHIRALPAK ID	Immobilized	
CHIRALPAK IE	Immobilized	
CHIRALPAK IF*	Immobilized	

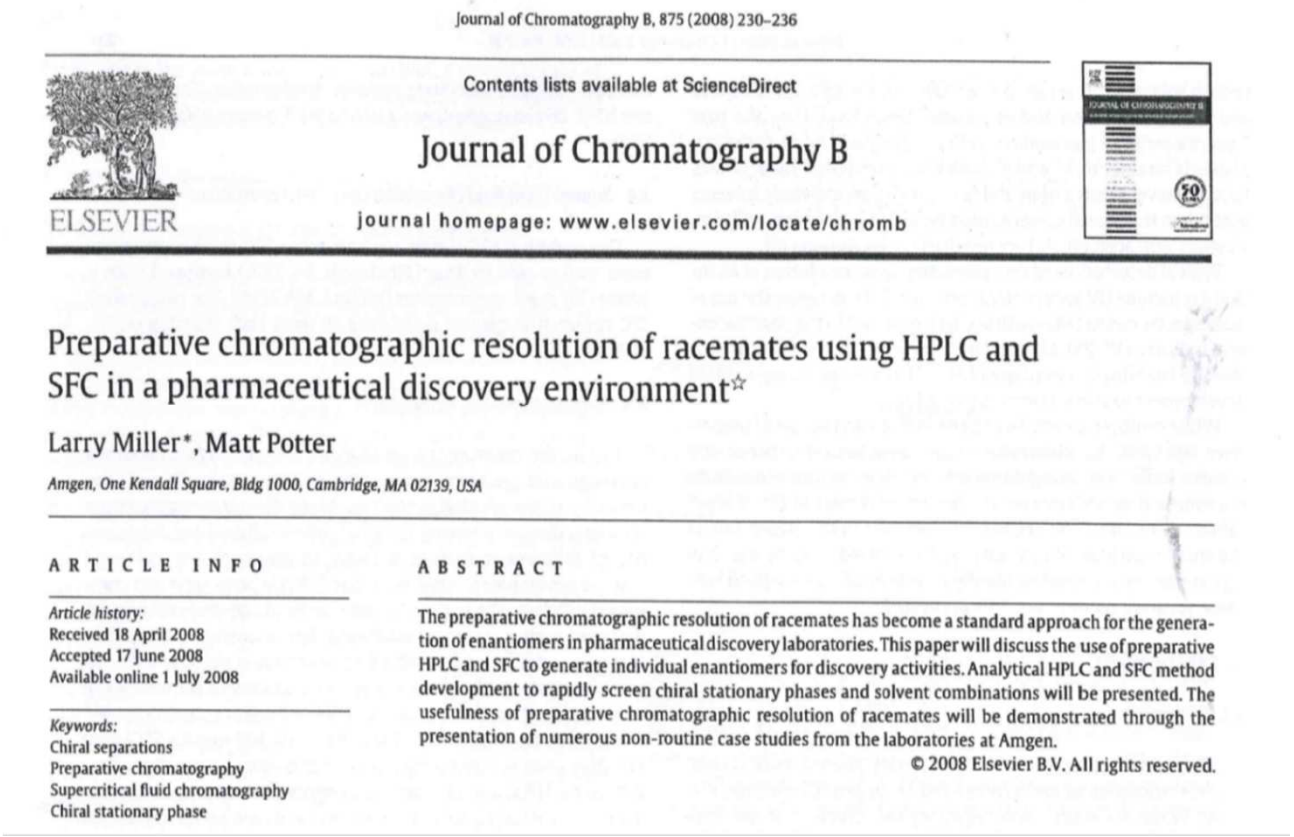


Cellulose-based

CSP	Nature	-R
CHIRALPAK IB	Immobilized	
CHIRALPAK IC	Immobilized	

** Not available when study was performed*

Gradient or isocratic?



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journal homepage: www.elsevier.com/locate/chromb

Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment☆

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ABSTRACT

The preparative chromatographic resolution of racemates has become a standard approach for the generation of enantiomers in pharmaceutical discovery laboratories. This paper will discuss the use of preparative HPLC and SFC to generate individual enantiomers for discovery activities. Analytical HPLC and SFC method development to rapidly screen chiral stationary phases and solvent combinations will be presented. The usefulness of preparative chromatographic resolution of racemates will be demonstrated through the presentation of numerous non-routine case studies from the laboratories at Amgen.

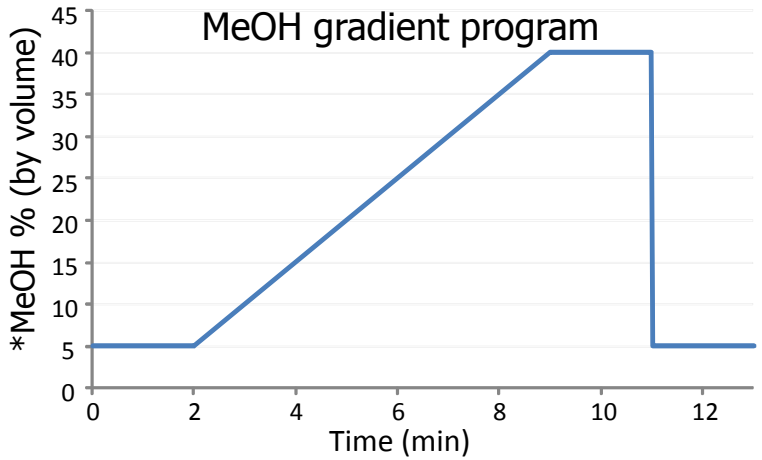
L. Miller et al., J. Chromatogr. B 875 (2008) 230-236

1. Combination of LC and SFC
2. Initial screening in gradient mode
3. Final isocratic method

Literature example

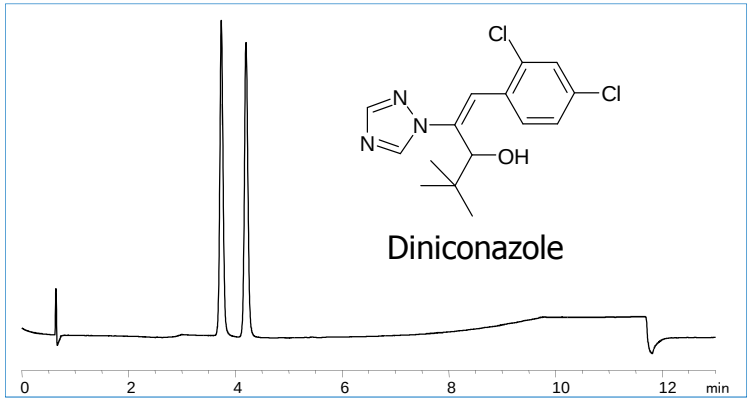
Gradient or isocratic?

Some examples in SFC



Back pressure: 150bar, Flow rate: 3.0ml/min,
Temperature: 35°C

* Containing 0.3% DEA for screening of basic compounds



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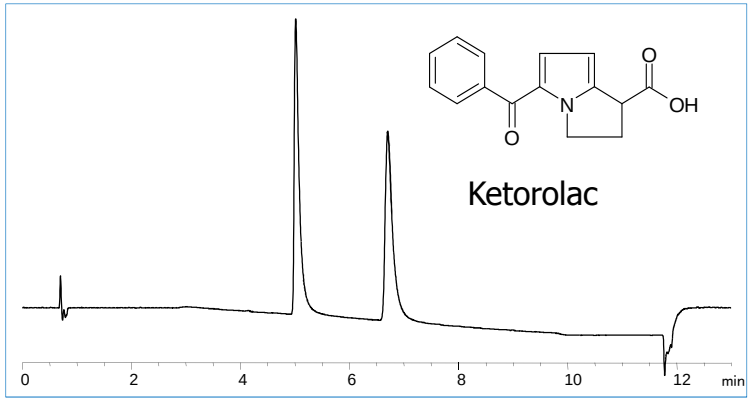
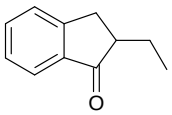


CHIRALPAK ID

From gradient to isocratic

2-Ethyl-1-indanone



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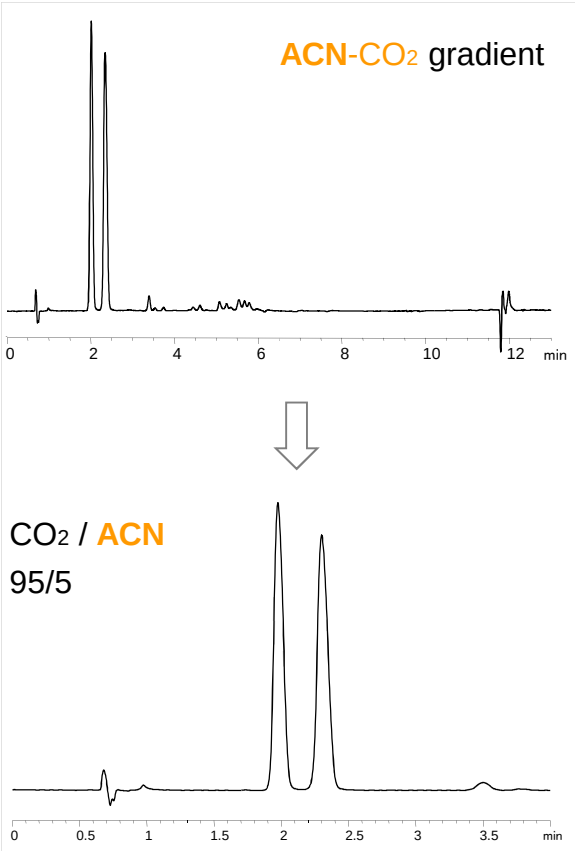
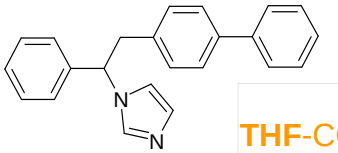


Bifonazole



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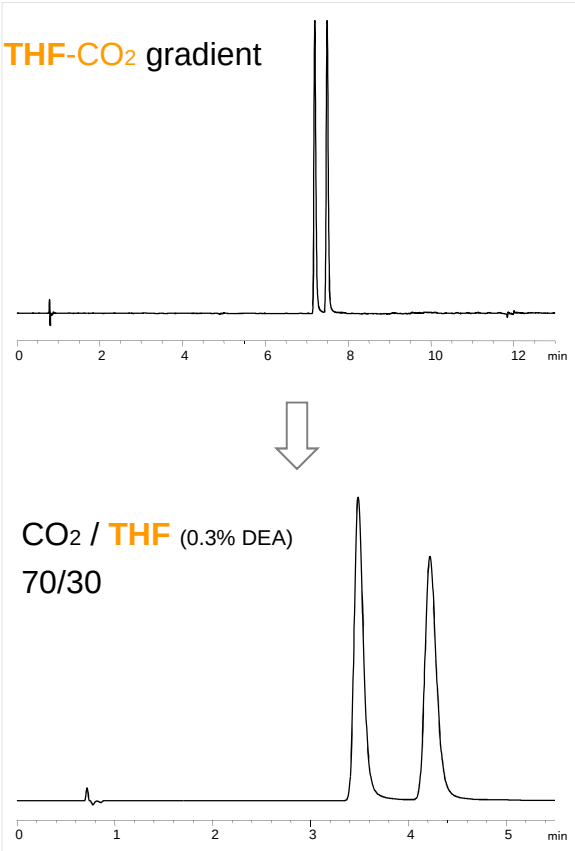


Easy transfer of conditions

SFC Conditions used in the study

System	Berger Analytical System
Column	IA , IB , IC , ID , IE
Column Size	250 x 4.6 mm, 5 µm
Mobile Phases	A: CO_2, B: Modifier (co-solvents) See next slide
Gradient	5 – 50% B gradient at 6.5%/min, hold for 3 mins, re-equilibrate for 2 mins
Temp.	40° C
Pressure	120 bar
Detection	DAD: 215, 230, 254 nm
Flow	4.0 mL/min
Injection	10 µL
Run Time	12 min

Evolution screening conditions in the study

Initial setup

- 100% THF
- 100% EtOAc
- 50% DCM/50% MeOH

Revised setup

- 95% THF/5% MeOH
- 90% EtOAc/10% MeOH
- 90% DCM/10% MeOH

• 0.1% IPAmine added to co-solvents for basic molecules

• 0.2% IPAmine added to co-solvents for basic molecules

•No acid additive for acidic molecules

• 0.2% TFA added to co-solvents for acidic molecules

Selected Acidic Molecules

Code #	ACIDS
acid 1	***trans*-2-Phenyl-1-cyclopropane carboxylic acid**
acid 2	**Bis-N-Cbz-DL-Lysine**
acid 3	**Sulindac**
acid 4	**Suprofen**
acid 5	**Proglumide**
acid 6	**Dichlorprop**
acid 7	**Fenoprofen, Ca-Salt**
acid 8	**2-(4-Hydroxy-phenyl)-propionic acid**
acid 9	**3-Phenylbutyric acid**
acid 10	**2-Phenylbutyric acid**
acid 11	**2-Phenylpropionic acid**

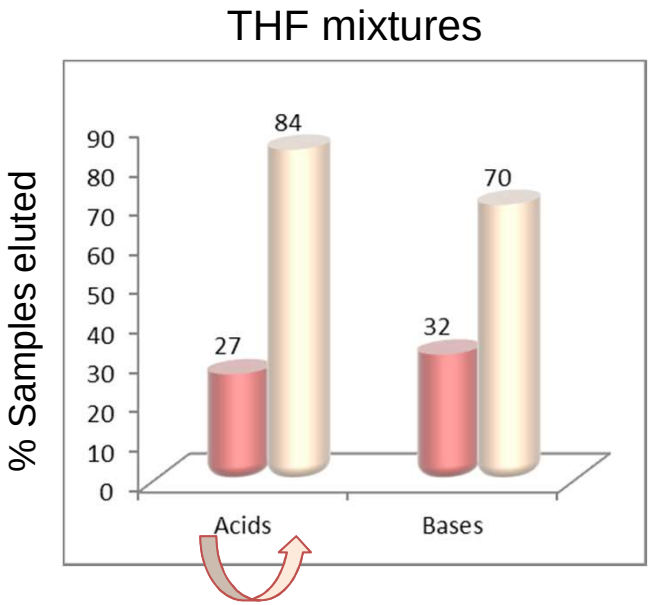
11 acids

Selected Basic Molecules

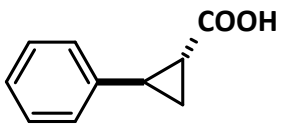
Sample Code	BASES
basic 1	**Mebeverine HCl**
basic 2	**Propafenone HCl**
basic 3	**Homatropine**
basic 4	**Labetalol HCl**
basic 5	**Verapamil HCl**
basic 6	**Chlophedianol HCl**
basic 7	**Acebutolol**
basic 8	**Atropine**
basic 9	**Ofloxacin (zwitterionic)**
basic 10	**Diperodon HCl**
basic 11	**Ketamine HCl**
basic 12	**Nadolol**
basic 13	**Salbutamol HCl**
basic 14	**p-Chloroamphetamine HCl**

14 bases

The elution strength

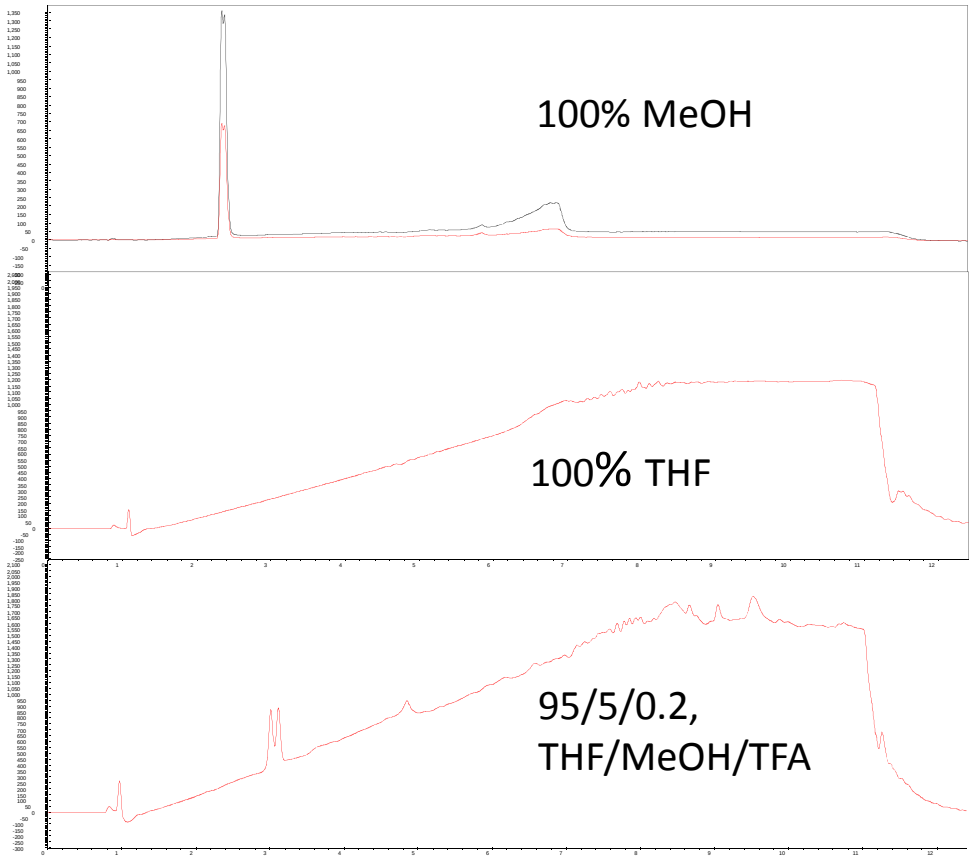


Addition of 10% MeOH to THF



Trans-2-phenyl-1-cyclopropane carboxylic acid

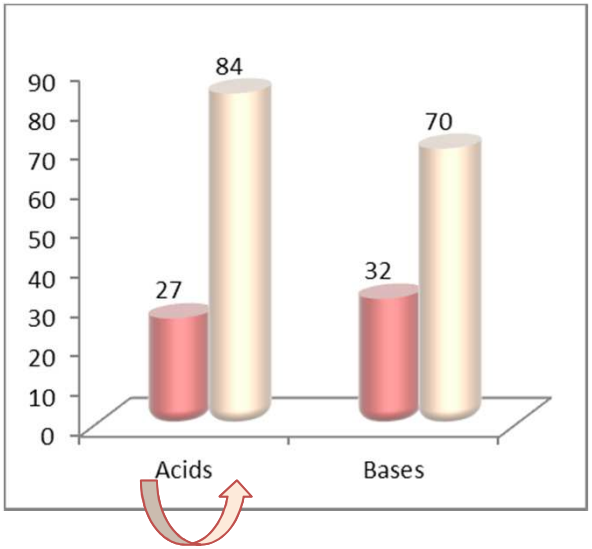
CHIRALPAK IA



The elution strength

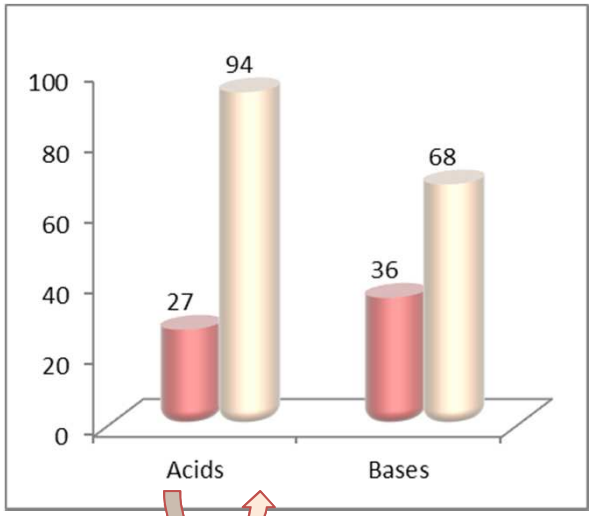
Percentages of compounds eluted

THF mixtures



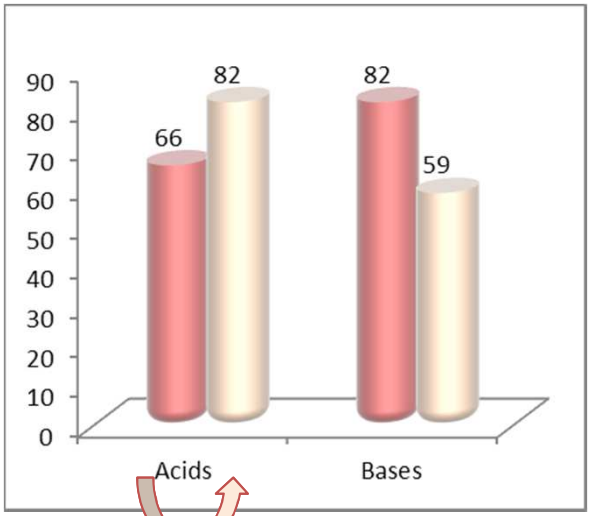
Addition of 10% MeOH to THF

EtOAc mixtures



Addition of 10% MeOH to EtOAc

DCM mixtures



From 50% MeOH to 10% MeOH in DCM

Statistics on all runs and conditions tested (total: 125)

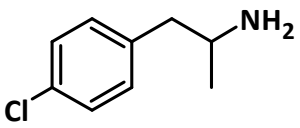
Bringing selectivity with extended solvent range

	ACIDS			
		Alcohols	Old Ext. run	New Ext. run
acid 1	trans-2-Phenyl-1-cyclopropane carboxylic acid	8	4	10
acid 2	Bis-N-Cbz-DL-Lysine	6	none eluted	3
acid 3	Sulindac	6	2	4
acid 4	Suprofen	8	4	12
acid 5	Proglumide	11	4	12
acid 6	Dichlorprop	2	3	8
acid 7	Fenoprofen, Ca-Salt	0 resolved	3	5
acid 8	2(4-HO-PheO)-propionic acid	5	4	13
acid 9	3-Phenylbutyric acid	1	1	6
acid 10	2-Phenylbutyric acid	0 resolved	0 resolved	4
acid 11	2-Phenylpropoionic acid	0 resolved	0 resolved	3
	BASES			
		Alcohols	Old Ext. run	New Ext. run
basic 1	Mebeverine HCl	9	4	6
basic 2	Propafenone HCl	9	5	7
basic 3	Homatropine	7	1	2
basic 4	Labetalol HCl	5	1	4
basic 5	Verapamil HCl	4	3	7
basic 6	Chlophedianol HCl	6	5	10
basic 7	Acebutolol	4	1	2
basic 8	Atropine	6	3	1 (IC)
basic 9	Ofloxacin	2	none eluted	1 (ID)
basic 10	Diperodon HCl	11	10	13
basic 11	Ketamin HCl	2	5	8
basic 12	Nadolol	12	3	1 (IA)
basic 13	Salmbutamol HCl	4	0 resolved	1 (IA)
basic 14	DL-p-Chloroamphetamine HCl	0 resolved	3	7

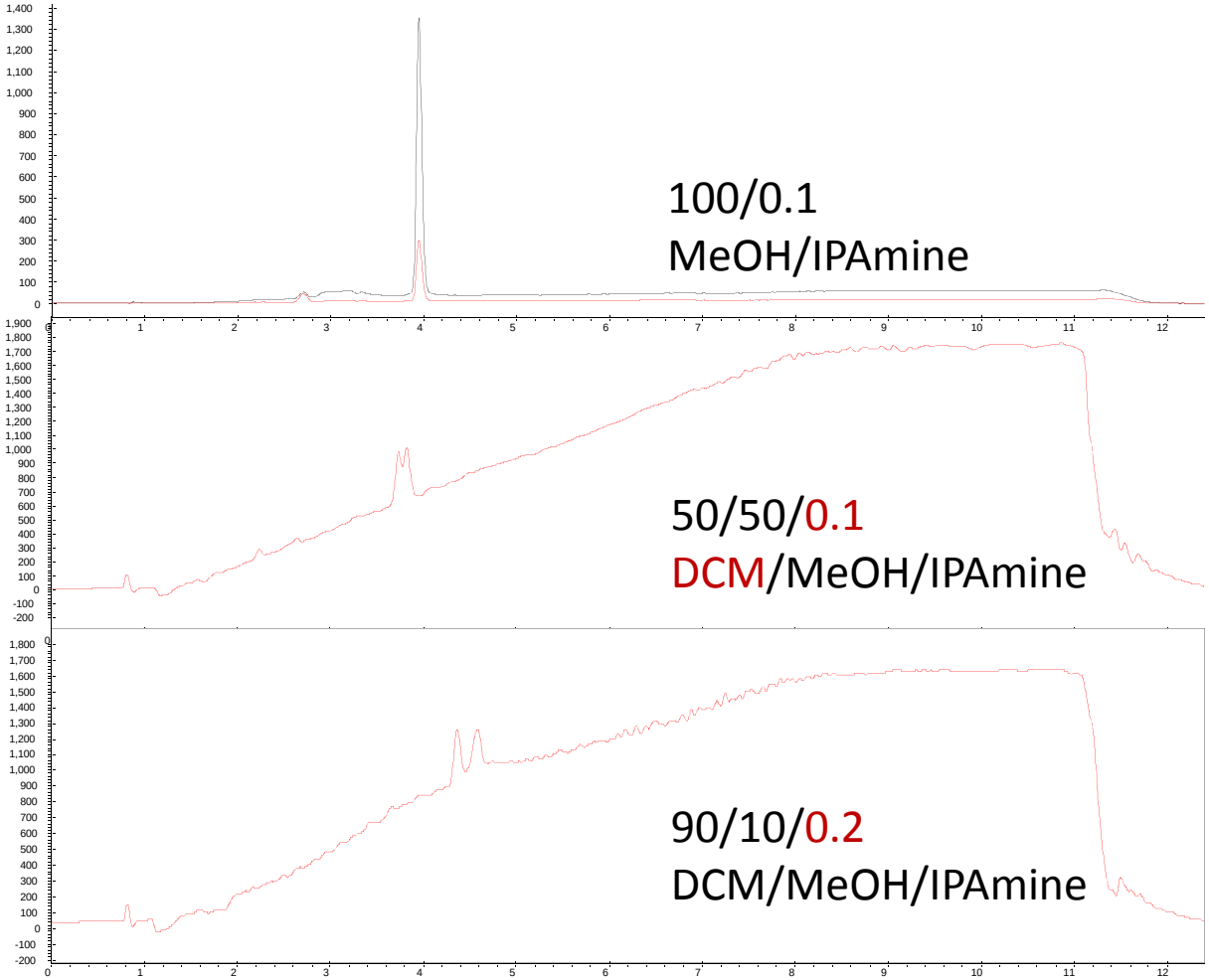
Number of runs where there was resolution observed

Bringing selectivity with extended solvent range

p-chloroamphetamine HCl

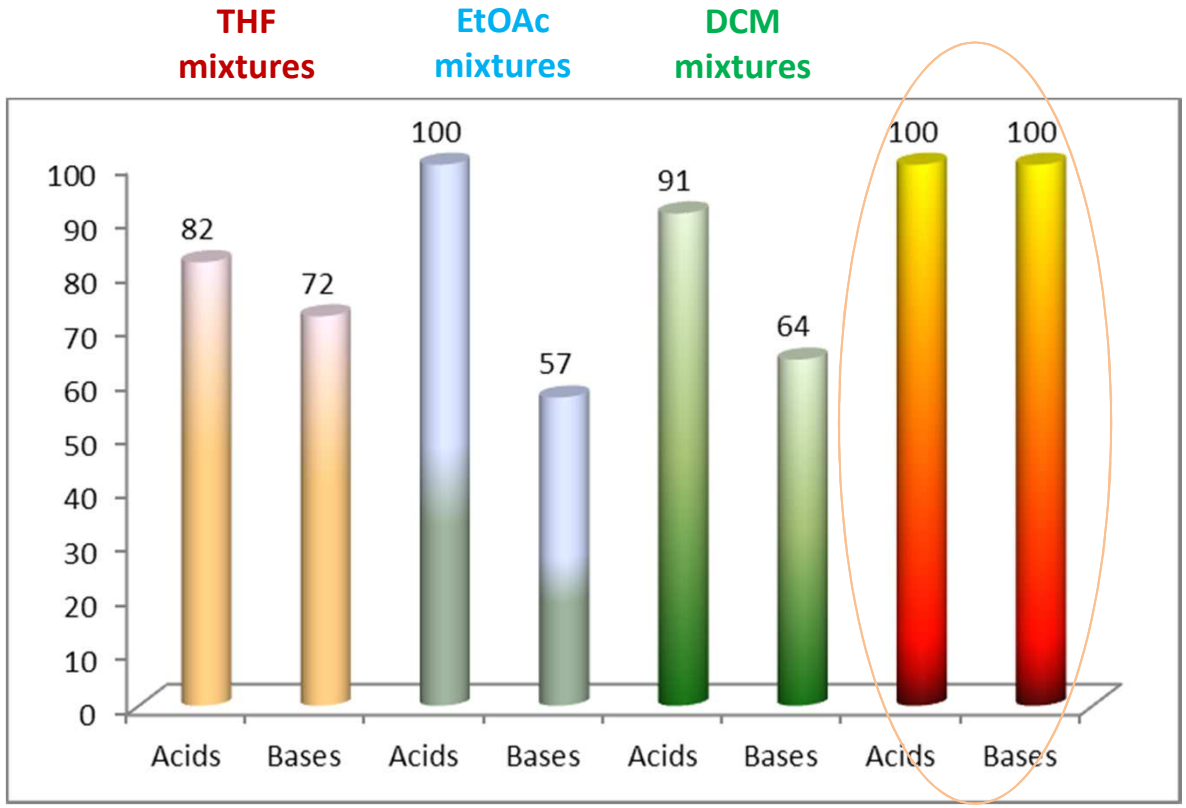


CHIRALPAK IC



Influence of
% basic additive

The success rate



Global success extended solvent range

- ✓ All molecules resolved in the extended solvent range
- ✓ For acidic compounds, EtOAc leads to higher success rate
- ✓ For bases, it may be important to further investigate higher % basic additive

Statistics on success rate per molecule

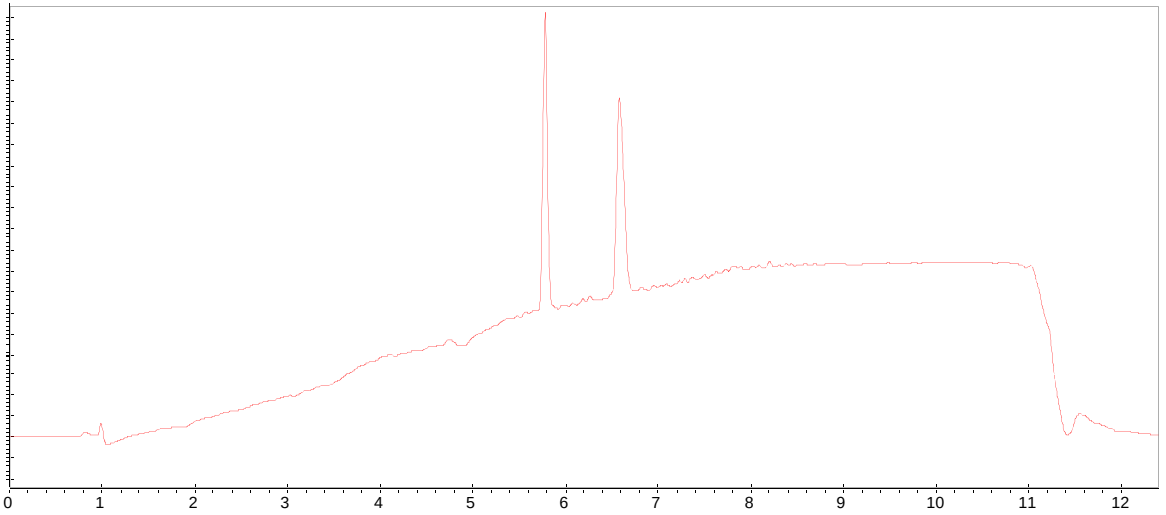
Overview of conditions tested - THF

THF / MeOH
90 /10
+
0.2 additive

- ✓ Proper elution strength (>70%)
- ✓ Resolution success rate:
  - ✓ 82% for acids
  - ✓ 72% for bases

Diperodon

CHIRALPAK IA
THF/MeOH/IPAmine



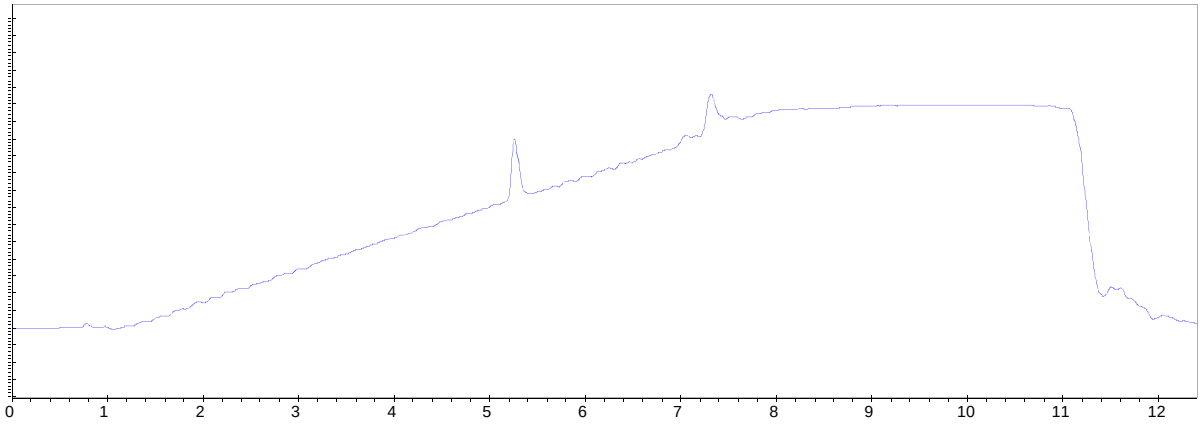
Overview of conditions tested - EtOAc

EtOAc / MeOH
90 /10
+
0.2 additive

- ✓ Proper elution strength (>70%)
- ✓ Resolution success rate:
  - ✓ 100% for acids
  - ✓ 57% for bases
- ✓ For bases, the addition of more alcohol and higher % IPAmine should be investigated

Verapamil

CHIRALPAK IB
EtOAc/MeOH/IPAmine

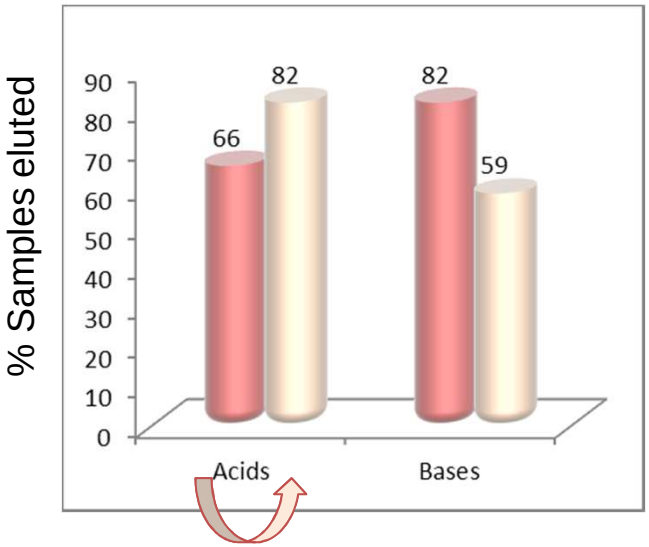


Overview of conditions tested - DCM

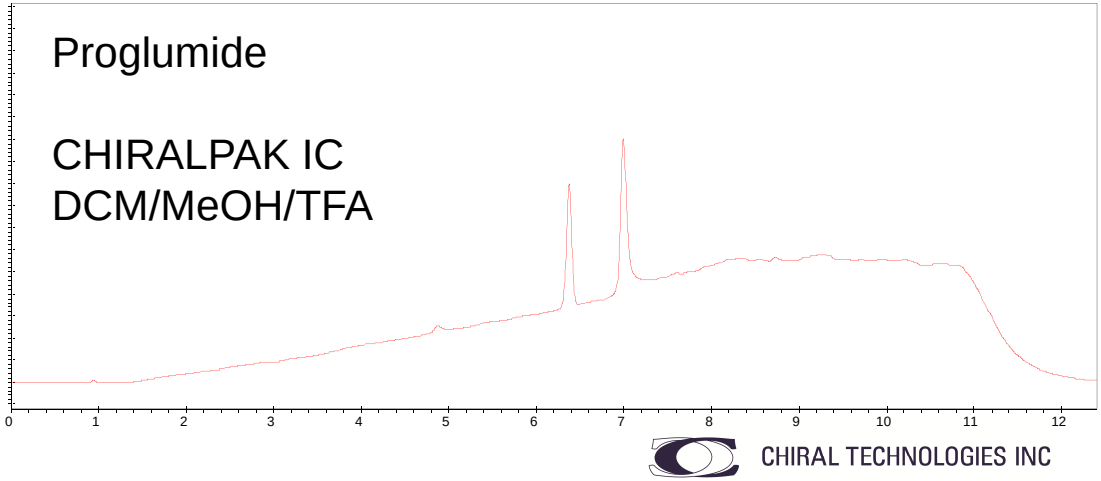
DCM/ MeOH
50 /50 or 90/10
+
0.2 additive

- ✓ Different tendencies for acids and bases
  - ✓ Acids elute better and reach higher success rates with 10% alcohol
  - ✓ Bases need more alcohol for elution and resolution
- ✓ Resolution success rate:
  - ✓ 91% for acids
  - ✓ 64% for bases
- ✓ For bases, the addition of more alcohol and higher %IPAmine should be investigated

DCM mixtures



From 50% MeOH to 10% MeOH in DCM



Column Performance Comparisons

Total 25 compounds (acids and bases)					
Co-solvents	IA	IB	IC	ID	IE
95% THF/5%MeOH	10	6	14	12	7
90% EtOAc/10%MeOH	13	11	13	12	9
90% DCM/10%MeOH	11	7	9	8	8
Total Hits	34	24	36	32	24
Co-solvents					
95% THF/5%MeOH	IC > ID > IA > IE > IB				
90% EtOAc/10%MeOH	IC = IA > ID > IB > IE				
90% DCM/10%MeOH	IA > IC > ID = IE > IB				
Totals	IC > IA > ID > IE = IB				

Conclusions

- ✓ The use of extended solvent range in SFC for chiral applications offers advantages to:
  - ✓ Increase solubility of the samples
  - ✓ Avoid instability of molecules sensitive to alcohols
  - ✓ Broaden selectivity profiles and increase success rate

    High interest to explore all analytical options of SFC now that new powerful SFC equipment is available

- ✓ Screening strategies should be adapted accordingly in terms of co-solvent composition and presence of additives

- ✓ Other solvents (MtBE, …) and conditions (other additive combinations) could be further explored

Acknowledgements

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